REMARKS

Upon entry of the amendment, claims 1-5, 7-8, 18-23, 52-54 and 63-64 will be pending in the application. Claims 25-30, 32-34, 36, 42-48, 51 and 55-62 are cancelled with the present amendment and new claims 63-64 added. Claims 1, 7, 8, 18-23 and 52 are amended. Support for the amendments to claim 1 appears in, e.g., the specification at Example 1, page 34, line 19 – page 43, line 7, Table 1 and Figures 1-4; more specifically at page 39, lines 19-24, and the paragraph bridging pages 32-33. Support for the amendments to claim 7 and new claims 63-64 appears in, e.g., the specification at page 6, lines 6-8; page 9, line 1-3; page 12, lines 13-15, 28-30; page 27, lines 4-10; page 39, lines 1-3; page 42, lines 23-24; Figure 18; claim 34). The remaining amendments more clearly point out the subject matter claimed, address various informalities, and/or clarify antecedent bases.

No new matter is added by these amendments. The cancellation of claimed subject matter of does not constitute an admission by Applicants that the subject matter no longer claimed is not patentable. Applicants reserve the right to pursue all cancelled subject matter in a continuing application or applications.

In support of the remarks and arguments stated *infra*, Applicants submit as Exhibit 1 a copy of Declaration signed by Dr. Eitan Fibach ("Fibach Declaration II") and submitted on April 22, 2004 in parent application USSN 09/463,320. [1]

Rejections under 35 U.S.C. § 112, First Paragraph

Claims 1-5, 7-8, 18-23 and 51-56 are rejected under 35 U.S.C. § 112, first paragraph for introducing new matter. The Examiner acknowledges that the specification supports a genus of hematopoietic cells and progenitor cells but states the specification does not support a subgenus of committed progenitor hematopoietic cells and/or non-differentiated, early hematopoietic progenitor cells as recited in claim 1. The rejection is traversed to the extent it is applied to the claims as amended.

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[1] Another Declaration signed by Dr. Fibach was submitted in the Response filed December 2, 2003 for the present application.

Applicants have amended claim 1, from which the remaining claim subject to the rejection depend, to delete committed progenitor hematopoietic cells and/or non-differentiated, early hematopoietic progenitor cells; and, Applicants have amended claim 7 and added claim 63 to recite committed progenitor hematopoietic cells and non-differentiated, early progenitor hematopoietic cells, respectively.

Applicants submit that the specification demonstrates that they were also in possession of the subgenus "committed progenitor hematopoietic cells and/or non-differentiated, early hematopoietic progenitor cells" when the application was filed. Applicants disclose in the specification that transition metal chelators promote proliferation while inhibiting differentiation of many cell types including preferably hematopoietic cells (*See* specification at page 8, line 27-28). Further, the specification discloses that in another preferred embodiment, the hematopoietic cells can be non-differentiated stem cells or committed progenitor cells (*See* specification at page 9, line 1-3; page 12, lines 13-15, 28; page 27, lines 4-10) or early progenitor cells (*See* specification at page 6, lines 6-8; page 39, lines 1-3; page 42, lines 23-24; Figure 18). This demonstrates that Applicants were in full possession of the invention as claimed.

In view of the foregoing comments, Applicants submit the pending claims meet the requirements under 35 U.S.C. § 112, first paragraph. Therefore, Applicants respectfully request that the rejection be withdrawn.

Rejections under 35 U.S.C. 103(a)

Claims 1-5, 7-8, 18-23, 25-30, 32-34, 36, 42-48 and 51-62 are rejected as obvious over Moore et al, Blood Cells, 20: 468-48, 1994 ("Moore"); or De Bruyn et al., Stem Cells 13: 281-288, 1995 ("De Bruyn"), each in view of Cicuttine et al. Blood 80: 102-112 (1992) ("Cicuttine") and Percival , J. Nutrition 122: 2424-2429 (1992) ("Percival I"). Claims 25-30, 32-34, 36, 42-48, 51 and 55-62 have been cancelled. The rejection is traversed to the extent it is applied to the pending claims as amended.

Independent claim 1, from which the remaining claim subject to the rejection depend, has been amended to recite an expanded hematopoietic cell population obtained *ex vivo* by providing

cells in culture with conditions for cell proliferation and a transition metal chelator and to specify that the transition metal chelator and conditions of proliferation result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state, thereby inhibiting differentiation while permitting expansion of the hematopoietic cell population.

As discussed in Applicants' previous response and detailed below, the combination of Moore or De Bruyn with either Cicuttine or Percival I is improper, relying on hindsight. Even if made (improperly), the suggested combinations do not (and cannot) teach or suggest the claimed expanded hematopoietic cell population obtained *ex vivo* by providing cells in culture with conditions for cell proliferation and a transition metal chelator and to specify that the transition metal chelator and conditions of proliferation result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state, thereby inhibiting differentiation while permitting expansion of the hematopoietic cell population. In addition, there are secondary considerations present here, including a solution to a long-felt but unsolved need, praise for the invention, and unexpected results. Graham v. John Deere Co., 383 U.S. 1, 17-18 (1966).

Secondary Considerations That Must Be Considered

There has been a long-felt but unsolved need for expanded hematopoietic cell populations obtained *ex vivo* (and particularly expanded subpopulations of clonogenic stem and progenitor populations therein). While expanded hematopoietic cell populations have been derived from bone marrow or stored umbilical-cord blood to reconstitute the immune systems of patients with leukemia or other hematologic cancers, these traditional expanded hematopoietic cell populations have typically not yielded sufficient quantities of hematopoietic cells to treat adult patients. The expanded hematopoietic cell population of the present invention solves this long-felt need by providing a greater expanded hematopoietic cell population and specifically of expanded cells with engraftment ability such as the stem and progenitor subpopulations (e.g. $CD34^+$ or AC133 cells and subsets) which increases the effectiveness of short-term and long-term engraftment when the expanded cell population is transplanted into patients. *See* Fibach Declaration II at ¶ 7.

Applicants' claimed expanded hematopoietic cell population requires the use of a transition metal chelator. The claimed expanded hematopoietic cell population shows an unexpectedly superior expansion of the hematopoietic population in total, as well as an unexpectedly superior selective expansion of the stem and progenitor populations therein (which is critical for short-term and long-term engraftment effectiveness of the expanded population).

As the specification demonstrates, the present invention teaches that the expanded hematopoietic cell population obtained *ex vivo* following incubation with a transition metal chelator in combination with early acting cytokines shows increased cell proliferation and also displays great increases in the expansion of the subpopulation of clonogenic cells (CFUc) hematopoietic cells obtained (representative of the stem and progenitor populations) and includes undifferentiated cells maintained in their undifferentiated state, as well as, cells inhibited from undergoing differentiation and (*See* specification at Example 1, page 34, line 19 – page 43, line 7, Table 1 and Figures 1-4). *See* Fibach Declaration II at ¶ 8.

Specifically, the addition of a transition metal chelator, such as TEPA, to cultures containing an early acting cytokine, such as IL-3, surprisingly resulted in a two-fold increase in total cells, four-fold increase in percentage of $CD34^+$ cells present in the total expanded cell population and, most strikingly, a five-fold increase in clonogenic cell (CFUc) expansion (*See* specification Table 1 at page 37, comparing rows 4 and 5). These results demonstrate that providing hematopoietic cells with a transition metal chelator led to excellent expansion of hematopoietic cells. Moreover, these results show the superior and preferential expansion of progenitor subsets such as clonogenic cells (CFUc) and $CD34^+$ cells. The fold expansion of progenitor cell subsets is higher than the fold expansion of total nuclear cells. Thus, progenitor cell frequency increased among the entire cell population. An expanded hematopoietic cell population displaying the enrichment and expansion of this subpopulation of hematopoietic cells, i.e., stem or progenitor cells, is critical to improving short-term and long-term engraftment of the expanded hematopoietic cells in transplantation and adoptive immunotherapy. *See* Fibach Declaration II at ¶ 9.

Moreover, the results in Example 1 also surprisingly demonstrate that a transition metal chelator with affinity for copper (e.g., TEPA) greatly improved clonability in long term cultures

of expanded cells, and in fact, the clonability of long term cultures surpass that of cells in short term cultures (*See* specification at Example 1, page 37, lines 12-15 and Figures 3-4). This data displays the superior properties of the claimed expanded hematopoietic cell population, since it is essential that self-renewal be maximally prolonged in order to achieve maximal *ex vivo* expansion of hematopoietic cells. *See* Fibach Declaration II at ¶ 9.

Recent studies, phase I human clinical trials, using the claimed expanded hematopoietic cell population have confirmed the results disclosed in Example 1 of the present invention. The trial comprised four human patients (3 Males, 1 Female) with varying diagnoses and ranging in age from 10-24 and in weight from 50-77 kg. In the trial, hematopoietic cells from donors were treated with a transitional metal chelator as described in the present invention to obtain an expanded hematopoietic cell population. These results confirm the surprising and unexpected results disclosed in Example 1. Specifically, the trials showed the obtained expanded hematopoietic cell population displayed superior expansion of not only Mononuclear Cells (MNC) (expansion from 74 to 420.5 fold) but also the striking and superior selective expansion of $CD34^+$ cells (expansion from 1.9 to 57.8 fold). More specifically, an expanded hematopoietic cell population from patient 1 showed a very good 2 fold expansion, patients 3 and 4 an excellent 20.9 and 29.6 fold expansion, respectively and an expanded hematopoietic cell population from patient 2 showed a dramatic 57.8 fold expansion of clonogenic cells (CFUc).

In sum, these results demonstrate that the claimed expanded hematopoietic cell population displays a superior expansion of clonogenic cells (CFUc), and that this in turn will result in greatly improved short-term and long-term engraftment effectiveness of the expanded hematopoietic cell population. *See* Fibach Declaration II at ¶ 10.

Finally, Applicants have received praise for the claimed methods. As Dr. Fibach states, a scientific Abstract reporting results obtained with the claimed expanded hematopoietic cell population received the "Best Abstracts Award" from the American Society for Blood and Marrow Transplantation. Moreover, the improved long-term engraftment effectiveness of the expanded hematopoietic cell population described in the instant invention has been praised in The Wall Street Journal health section. *See* Fibach Declaration II at ¶ 11.

The combination of either of the primary references, Moore or De Bruyn, with either of Cicuttine or Percival I could not lead the ordinarily skilled artisan to the solution to the long-felt need (an *ex vivo* expanded hematopoietic cell population, particularly expanded subpopulations of clonogenic stem and progenitor populations therein and the resulting improved short-term and long-term engraftment of these expanded cells), nor to the unexpected and superior advantages (prolonged active cell proliferation, prolonged expansion of clonogenic cells (CFUc) and maintenance of undifferentiated cells in their undifferentiated state) that the claimed invention provides.

The Combinations Are Improper And In Any Event Are Defective

The combination of either of the primary references with either of the secondary references is improper – the ordinarily skilled artisan is not directed to the suggested combinations (for reasons detailed below) and the combinations can only be made with the impermissible application of hindsight.

Determination of obviousness cannot be based on the hindsight combination of components selectively culled from the prior art to fit the parameters of the patented invention. Crown Operations Int'l, LTD v. Solutia Inc., 289 F.3d 1367 (Fed. Cir. 2002). In making the assessment of differences, 35 U.S.C. § 103 specifically requires consideration of the claimed invention "as a whole", it being well recognized that inventions typically are new combinations of existing principles or features. Envtl. Designs, Ltd. v. Union Oil Co., 713 F.2d 693 (Fed. Cir. 1983). The "as a whole" instruction in 35 U.S.C. § 103 prevents evaluation of the invention part by part, such that the obviousness assessment improperly breaks an invention into its component parts (A + B + C), then uses a prior art reference containing A, another containing B, and another containing C, and on that basis alone declare the invention obvious. Ruiz v. A.B. Chance Co., 357 F.3d 1270 (Fed. Cir. 2004).

The Examiner concedes that neither Moore nor De Bruyn explicitly teach the claimed expanded hematopoietic cell population, with the express requirements that the hematopoietic cells are obtained *ex vivo* by providing the hematopoietic cells with a transition metal chelator

having an affinity for copper such that the proliferation conditions and presence of chelator result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state.

The Examiner has then applied two secondary references, Cicuttine or Percival I, to supply the claimed requirement for a transition metal chelator with affinity for copper. The Examiner has already conceded that the primary references Moore and De Bruyn do not teach or suggest an expanded hematopoietic cell population obtained by providing a transition metal chelator, and certainly not the specifically recited transition metal chelator with an affinity for copper (*See* Office Action at page 4, second paragraph).

There is nothing in Cicuttine that would motivate the ordinary skilled artisan to combine its teachings with either of the primary references Moore or De Bruyn to arrive at the claimed invention. Cicuttine does not cure the deficiencies of Moore or De Bruyn; as Cicuttine does not teach or suggest an expanded hematopoietic cell population obtained by providing a transition metal chelator with an affinity for copper and proliferation conditions that result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state, as specifically required by the amended claims.

The ordinarily skilled artisan would not combine Cicuttine with either of the primary references for a variety of reasons.

Cicuttine refers to the control of proliferation of stromal cell lines that are in turn co-cultured with certain hematopoietic cells – Zinc was added to the culture medium only to switch on the T oncogene under the control of a Zn-responsive element of a metallothionein promoter relating to the proliferation of the underlying stromal cell line (*See* Cicuttine at page 103, column 1, lines 25-28). Moreover, the specified culture conditions exclude the possibility that the hematopoietic and stroma cell co-cultures are initiated in the presence of zinc as the zinc is specifically washed out prior to the introduction of hematopoietic cells to the co-culture (*See* Cicuttine at page 104, column 2, lines 7-12). *See* Fibach Declaration II at ¶ 13.

Cicuttine does not refer in any way to an expanded hematopoietic cell population obtained by providing a transition metal chelator with an affinity for copper in the culture of hematopoietic cells for (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state. The Examiner states that "zinc has an affinity to copper and thus would reduce copper utilization of culturing hematopoietic cells". The Examiner concludes that it would have been obvious to one of ordinary skill in the art that culturing the cell in the medium containing zinc would reduce a capacity of hematopoietic cells in utilizing copper. *See* Office Action at page 6, third paragraph. The Examiner is apparently taking the position that Zinc is a transition metal chelator with an affinity for copper. As Dr. Fibach states in his Declaration, zinc is a transition metal and not a transition metal chelator with an affinity for copper, as required by the claimed methods. Moreover, as stated *supra*, the hematopoietic cells are not cultured in a medium containing zinc as suggested by the Examiner. *See* Fibach Declaration II at ¶ 13.

Further, Applicants have carefully reviewed the discussion section of Cicuttine and do not agree with the Examiner that there is support for the suggestion that zinc has an affinity for copper; in fact, the term copper is not disclosed in Cicuttine. As Dr. Fibach concludes, since both zinc and copper ions are positively charged species in solution, "affinity" is not likely. *See* Fibach Declaration II at ¶ 13.

The use of the isolated statements in Cicuttine in combination with the primary references is impermissible hindsight -- Cicuttine's use of Zinc to control proliferation of a metal sensitive promoter driving oncogenic proliferation of a stromal cell line (and not the hematopoietic cells directly or indirectly) cannot provide the requisite teaching to support combination with the primary references here.

In addition, even if the combination were proper (it is not) the combination would not lead to the claimed invention – that is because (a) there is not one word in the primary references about use of a transition metal chelator, and (b) nor is there a single word in Cicuttine regarding the use of a transition metal chelator, as expressly required by the claims. Nor is there any teaching or suggestion that use of a transition metal chelator would provide the surprising and unexpected results detailed above and in Dr. Fibach's declaration. The combination must fail.

For these reasons, the ordinarily skilled artisan would not and could not combine Moore or De Bruyn with Cicuttine to reach the claimed invention.

In addition, the ordinary skilled artisan would not combine either Moore or De Bruyn with Percival I to reach the claimed invention. Even if the combination were proper (it is not), Percival I does not cure the deficiencies of Moore or De Bruyn.

There are many deficiencies in Percival I that the ordinarily skilled artisan would recognize, detailed below, which would teach away from combination with the primary references.

First, Applicants and the Examiner differ on interpretation of the claim language and certain language in Percival I. Specifically, Percival I states:

> In summary, incubating HL-60 cells with TEPA resulted in copper-deficient cells without loss of viability or alteration in the stage of differentiation. (*See* Percival I at page 2428, column 2, lines 34-36)

The Examiner concludes that this statement supports the Examiner's conclusion that Percival I teaches culturing conditions using defined growth medium conditions that will stimulate growth while inhibiting differentiation and further that chelating copper with TEPA will inhibit differentiation (*See* Office Action at page 4, fourth paragraph – page 5, first paragraph).

Applicants disagree with this characterization of Percival I. This statement in Percival I is correctly interpreted to mean that TEPA had no effect on the differentiation of the single cell line – HL-60 cells – used in Percival I. Other statements in Percival I clearly support this interpretation. The authors of Percival I hypothesized that TEPA may be an inducer of differentiation, and specifically test TEPA to determine if TEPA acts as an inducer of HL-60 differentiation:

> It was necessary ... to determine whether incubating the HL-60 cells with the copper-chelating compound would result in differentiation ... [i]ncubating cells with TEPA did not affect the respiratory burst activity, demonstrating that neither

the compound nor the chelation of copper resulted in cell differentiation. (*See* Percival I at page 2428, column 1, lines 22-34). *See* Fibach Declaration II at ¶ 14.

Applicants direct the Examiner to a later publication by Percival (Am. J. Clin. Nutr. 67:1064-68, 1998)("Percival II"). Percival II is crystal clear on the results of Percival I (noted as reference 27 in Percival II).[2]

Moreover, Percival I teaches nothing or teaches away (nor could it since it refers only to HL-60 cells) with regard to (i) prolonged active cell proliferation; and (ii) prolonged expansion of clonogenic cells (CFUc), as expressly recited in the amended claims. The differences between these express recitations in the claims and the disclosure in Percival I are detailed below:

(i) prolonged active cell proliferation (Percival I teaches away, stating that for HL-60 cells, TEPA "did not affect the growth rate" – i.e., expansion as unaffected by a transition metal chelator);

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[2] Percival II is quite clear – it first demonstrates that "copper supplementation enhanced retinoic acid-induced differentiation [of HL-60 cells]" (*See* Percival II at page 1066S, column 2, lines 5-6, referring to the studies of Bae and Percival, J. Nutrition 123: 997-1002 (1992)) and then based on that finding Percival II asks the following question: "If copper is removed from the cell is differentiation impaired or prevented?" (*See* Percival II at page 1066S, column 2, lines 7-8). The author of Percival II hypothesizes, similarly as the Examiner, stating "[w]e hypothesized that if copper is essential for differentiation, then chelation of copper with TEPA should prevent the cells from differentiating." (*See* Percival II at page 1066S, column 2, lines 8-11). Percival II then cites the studies of Percival I showing that this hypothesis is incorrect stating, "Cells incubated with TEPA and retinoic acid produced the same amount of superoxide anion as did the cells with retinoic acid, **indicating that differentiation had occurred**." (Emphasis Added) (*See* Percival II at page 1066S, column 2, lines 15-18). Percival II further states that "So whereas our TEPA model is useful in some studies related to manipulating copper concentrations and Cu/Zn SOD activity, **it does not prevent HL-60 cells from differentiating**." (Emphasis Added) (*See* Percival II at page 1066S, column 2, lines 28-30). This teaches away. In fact, it teaches precisely the opposite result achieved with the claimed methods. In fact, the inability of TEPA to inhibit differentiation prompted the author to develop a different model to study copper's role in the differentiation of HL-60 cells, "The lack of effect of TEPA on HL-60 differentiation prompted us to develop a mouse model to continue our investigation of copper's role in granulopoiesis." (*See* Percival II at page 1066S, column 2, lines 31-33). *See* Fibach Declaration ¶ 15.

(ii) prolonged expansion of clonogenic cells (CFUc) (Percival I teaches away, not referring to this population of cells at all, and referring only to HL-60 cells, whose expansion, unlike the claim recited population, was unaffected by a transition metal chelator. HL-60 is an immortalized cell line, which proliferates continuously without undergoing terminal differentiation under normal culture conditions (such as provided in Percival I), unless supplemented with a specific differentiation inducer such as retinoic acid. Moreover, the HL-60 cell line of Percival I was derived from a patient with acute promyelocytic leukemia, cannot be isolated from a normal donor or another patient, nor can it be introduced into a human host because of its leukemic nature). *See* Fibach Declaration II at ¶ 16.

In sum, for the above reasons, the ordinarily skilled artisan would not have combined either of the primary references with Percival I, especially since Percival I either does not teach at all, or teaches away on virtually every aspect of the expressly recited claim limitations.

Finally, the combination of either of the primary references, Moore or De Bruyn, with Percival I could not lead the ordinarily skilled artisan to the solution to the long-felt need (an *ex vivo* expanded hematopoietic cell population, particularly expanded subpopulations of clonogenic stem and progenitor populations therein and the resulting improved short-term and long-term engraftment of these expanded cells), nor to the unexpected and superior advantages (prolonged active cell proliferation, prolonged expansion of clonogenic cells (CFUc) and maintenance of undifferentiated cells in their undifferentiated state) that the claimed invention provides (as detailed above).

The § 103 rejections should be withdrawn.

On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below. A petition for a three-month extension of time and fee accompany this response. Applicants also enclose a Request for Continued Examination and the required fee. The Commissioner is authorized to charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 24024-501 CON 2A.

Respectfully submitted,

Dated: August 23, 2004

Reg. No. 41,874

Ivor R. Elrifi, Reg. No. 39,529
Attorney for Applicants
c/o MINTZ, LEVIN
Tel: (617) 542-6000
Fax: (617) 542-2241
Customer No. 30623

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Peled et al.		
SERIAL NUMBER:	09/463,320	EXAMINER:	Michail A. Belyavskyi, Ph.D.
FILING DATE:	January 22, 2000	ART UNIT:	1644
FOR:	METHODS OF CONTROLLING PROLIFERATION AND DIFFERENTIATION OF STEM AND PROGENITOR CELLS		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. EITAN FIBACH UNDER 37 C.F.R. §1.132

1. I am a co-inventor, together with Tony Peled and Avi Treves, of the subject matter claimed in the above-referenced U.S. patent application.

2. I received my Ph. D. degree from the Weizmann Institute of Science in Rehovot, worked as a post-doctoral fellow at the Cancer Research Center, College of Physicians and Surgeons of Columbia University, New York, NY, USA, and was a visiting scientist at the Laboratory of Chemical Biology, NIDDK, National Institutes of Health, Bethesda, MD, USA.

3. I am presently employed as researcher at the Hadassah University Hospital, Department of Hematology, where I am a full professor. My research focuses on the development of hematopoietic cells. Since the beginning of my career, I have published more than 150 scientific articles, more than 40 on various aspects of HL-60 cells, in highly regarded journals and books, and have presented my achievements at many international scientific conferences. I am a member of American Society of Hematology and The International Society of Experimental Hematology. I have served on the editorial board of the journal "Experimental Hematology".

4. I have reviewed the Office Action dated February 9, 2004. I understand that claims

1-2, 4-13, 15, 37-45 and 47 claims 1-2, 4-5, 7-13, 15, 37, 39, 42-45 and 47-57 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Moore et al, Blood Cells, 20: 468-48, 1994 ("Moore"); or De Bruyn et al., Stem Cells 13: 281-288, 1995 ("De Bruyn"), each in view of Cicuttine et al. Blood 80: 102-112 (1992) ("Cicuttine") and of Percival , J. Nutrition 122: 2424-2429 (1992) ("Percival I").

5. I have reviewed the present application in conjunction with the Moore, De Bruyn, Cicuttine and Percival I references.

6. The claims as amended require providing hematopoietic cells *ex vivo* with a transition metal chelator having an affinity for copper and proliferation conditions which result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state.

7. There has been a long-felt but unsolved need for methods that permit *ex vivo* expansion of hematopoietic cells (and particularly subpopulations of clonogenic stem and progenitor populations therein). Hematopoietic cells are currently routinely expanded from bone marrow or stored umbilical-cord blood to reconstitute the immune systems of patients with leukemia or other hematologic cancers. Traditional methods of hematopoietic cell expansion have typically not yielded sufficient quantities of hematopoietic cells to treat adult patients. The methods of the present invention solves this long-felt need by providing greater expansion of hematopoietic cell populations and specifically of cells with engraftment ability such as the stem and progenitor subpopulations (e.g. $CD34^+$ or AC133 cells and subsets) which increases the effectiveness of short-term and long-term engraftment when the expanded population is transplanted into patients.

8. The present invention teaches that providing hematopoietic cells with a transition metal chelator in combination with early acting cytokines results in not only an inhibition of differentiation and increased cell proliferation but greatly increases the expansion of a subpopulation of clonogenic cells (CFUc) (representative of the stem and progenitor populations) and maintains undifferentiated cells in their

undifferentiated state (*See*, specification at Example 1, page 34, line 8 – page 42, line 33, Table 1 and Figures 1-4).

9. Specifically, the addition of a transition metal chelator, such as TEPA, to cultures containing an early acting cytokine, such as IL-3, surprisingly resulted in a two-fold increase in total cells, four-fold increase in percentage of $CD34^+$ cells present in the total cell population and, most strikingly, a five-fold increase in clonogenic cell (CFUc) expansion (*See*, specification Table 1 at page 36, comparing rows 4 and 5). These results demonstrate that providing hematopoietic cells with a transition metal chelator led to excellent expansion of hematopoietic cells. Moreover, these results show the superior and preferential expansion of progenitor subsets such as clonogenic cells (CFUc) and $CD34^+$ cells. The fold expansion of progenitor cell subsets is higher than the fold expansion of total nuclear cells. Thus, progenitor cell frequency increased among the entire cell population. The enrichment and expansion of this subpopulation of hematopoietic cells, i.e., stem or progenitor cells, is critical to improving short-term and long-term engraftment in hematopoietic cell transplantation and adoptive immunotherapy.

   Moreover, the results in Example 1 also surprisingly demonstrate that a transition metal chelator with affinity for copper (e.g., TEPA) greatly improved clonability in long term cultures, and in fact, the clonability of long term cultures surpass that of cells in short term cultures (*See*, specification at Example 1, page 37, lines 4-7 and Figures 3-4). This data displays the superior properties of the claimed methods, since it is essential that self-renewal be maximally prolonged in order to achieve maximal *ex vivo* expansion of hematopoietic cells.

10. Recent studies, phase I human clinical trials, have confirmed and expanded on the results disclosed in Example 1 of the present invention. The trial comprised four human patients (3 Males, 1 Female) with varying diagnoses and ranging in age from 10-24 and in weight from 50-77 kg. In the trial, hematopoietic cells from donors were treated with a transitional metal chelator as described in the present invention. These results strengthened the surprising and unexpected results disclosed in Example 1. Specifically, the trials showed the superior expansion of not only Mononuclear Cells (MNC) (expansion from 74 to 420.5 fold) but also the striking

and superior selective expansion of $CD34^+$ cells (expansion from 1.9 to 57.8 fold). More specifically, patient 1 showed a very good 2 fold expansion, patients 3 and 4 an excellent 20.9 and 29.6 fold expansion, respectively and patient 2 a dramatic 57.8 fold expansion of clonogenic cells (CFUc).

In sum, these results demonstrate that the claimed methods result in the superior expansion of clonogenic cells (CFUc), and that this in turn will result in greatly improved short-term and long-term engraftment effectiveness (*See*, Appendix A).

11. The present invention has also received praise in receiving the "Best Abstracts Award" from the American Society for Blood and Marrow Transplantation and has been praised in The Wall Street Journal health section for the improved long-term engraftment effectiveness of hematopoietic cells expanded by the process described in the claimed methods (*See*, Appendix B).

12. Moore and De Bruyn both refer to hematopoietic umbilical cord blood derived progenitor cells obtained from a donor. Neither Moore nor De Bruyn explicitly teach or suggest methods of transplanting hematopoietic cells or methods of adoptive immunotherapy by obtaining hematopoietic cells from a donor, providing the hematopoietic cells *ex vivo* with a transition metal chelator having an affinity for copper where the chelator and conditions result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state; thereby expanding the cells and transplanting the cells to a patient.

13. Cicuttine refers to the control of proliferation of stromal cell lines that are in turn co-cultured with certain hematopoietic cells – Zinc was added to the culture medium only to switch on the T oncogene under the control of a Zn-responsive element of a metallothionein promoter relating to the proliferation of the underlying stromal cell line (*See*, Cicuttine at page 103, column 1, lines 25-28). Moreover, the specified culture conditions exclude the possibility that the hematopoietic and stroma cell co-cultures are initiated in the presence of zinc as the zinc is specifically washed out prior to the introduction of hematopoietic cells to the co-culture (*See*, Cicuttine at page 104, column 2, lines 7-12). Cicuttine does not refer in any way to use of a

transition metal chelator with an affinity for copper in the culture of hematopoietic cells for (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state. The Examiner states that "zinc has an affinity to copper and thus would reduce copper utilization of culturing hematopoietic cells". The Examiner concludes that "it would have been obvious to one of ordinary skill in the art ... that culturing the cell in the medium containing zinc would reduce a capacity of hematopoietic cells in utilizing copper". *See*, Office Action at page 4, fifth paragraph. The Examiner is apparently taking the position that Zinc is a transition metal chelator with an affinity for copper. Zinc is a transition metal and not a transition metal chelator with an affinity for copper, as required by the claimed methods. Further, since both zinc and copper ions are positively charged species in solution, "affinity" is not likely. Moreover, as stated *supra*, the hematopoietic cells are not cultured in a medium containing zinc as suggested by the Examiner. Finally, there is no support in Cicuttine for the suggestion that zinc has an affinity for copper; in fact, the term copper is not disclosed in Cicuttine.

14. Percival I refers to a method of incubating of HL-60 cells with TEPA which results in reduced copper levels. Percival I does not teach or suggest "culturing conditions using defined growth medium conditions that will stimulate growth while inhibiting differentiation" (*See*, Office Action at page 5, first paragraph, lines 1-2) and that "chelating copper with TEPA will inhibit differentiation" (*See*, Office Action at page 5, first paragraph, lines 5-6) as suggested by the Examiner. Percival I is correctly interpreted to mean that TEPA had no effect on the differentiation of the single cell line – HL-60 cells – used in Percival I. Other statements in Percival I clearly support this interpretation. The authors of Percival I hypothesized that TEPA may be an inducer of differentiation, and specifically test TEPA to determine if TEPA acts as an inducer of HL-60 differentiation: "It was necessary ... to determine whether incubating the HL-60 cells with the copper-chelating compound would result in differentiation ... [i]ncubating cells with TEPA did not affect the respiratory burst activity, demonstrating that neither the compound nor the chelation of copper resulted in cell differentiation." (*See*, Percival I at page 2428, column 1, lines 22-34).

15. I have also reviewed a later publication by Percival (Am. J. Clin. Nutr. 67:1064-68, 1998)("Percival II"), which clarifies, comments on and expounds on the results of Percival I (noted as reference 27 in Percival II). Percival II is quite clear – it first demonstrates that "copper supplementation enhanced retinoic acid-induced differentiation [of HL-60 cells]" (*See*, Percival II at page 1066S, column 2, lines 5-6, referring to the studies of Bae and Percival, J. Nutrition 123: 997-1002 (1992)) and then based on that finding Percival II asks the following question: "If copper is removed from the cell is differentiation impaired or prevented?" (*See*, Percival II at page 1066S, column 2, lines 7-8). The author of Percival II hypothesizes, similarly as the Examiner, stating "[w]e hypothesized that if copper is essential for differentiation, then chelation of copper with TEPA should prevent the cells from differentiating." (*See*, Percival II at page 1066S, column 2, lines 8-11). Percival II then cites the studies of Percival I showing that this hypothesis is incorrect stating, "Cells incubated with TEPA and retinoic acid produced the same amount of superoxide anion as did the cells with retinoic acid, **indicating that differentiation had occurred**." (Emphasis Added) (*See*, Percival II at page 1066S, column 2, lines 15-18). Percival II further states that "So whereas our TEPA model is useful in some studies related to manipulating copper concentrations and Cu/Zn SOD activity, **it does not prevent HL-60 cells from differentiating**." (Emphasis Added) (*See*, Percival II at page 1066S, column 2, lines 28-30). This teaches away. In fact, it teaches precisely the opposite result achieved with the claimed methods. In fact, the inability of TEPA to inhibit differentiation prompted the author to develop a different model to study copper's role in the differentiation of HL-60 cells, "The lack of effect of TEPA on HL-60 differentiation prompted us to develop a mouse model to continue our investigation of copper's role in granulopoiesis." (*See*, Percival II at page 1066S, column 2, lines 31-33).

16. In reading Percival I and Percival II, it is clear that Percival I does not teach (i) prolonged active cell proliferation as Percival I states that for HL-60 cells, TEPA "did not affect the growth rate" – i.e., expansion was unaffected by a transition metal chelator and (ii) prolonged expansion of clonogenic cells (CFUc) as Percival I does not refer to this population of cells at all. Percival I refers only to HL-60 cells, whose expansion, unlike the claim recited population, was unaffected by a transition metal

chelator. HL-60 is an immortalized cell line, which proliferates continuously without undergoing terminal differentiation under normal culture conditions (such as provided in Percival I), unless supplemented with a specific differentiation inducer such as retinoic acid. Moreover, the HL-60 cell line of Percival I was derived from a patient with acute promyelocytic leukemia, cannot be isolated from a normal donor or another patient, nor can it be introduced into a human host because of its leukemic nature.

17. It is my view that the combination (and I do not believe this to be a proper combination) of Moore or De Bruyn with either Cicuttine or Percival I would not direct the ordinary skilled artisan to the unexpected results achieved with the claimed invention – namely prolonged active cell proliferation and prolonged expansion of a subset of hematopoietic cells, i.e. clonogenic cells (CFUs) -- and further would not direct the artisan to the unexpected, enhanced clonability of long term cultures. I am of the view that the present claimed methods of transplanting hematopoietic cells or methods of adoptive immunotherapy are not obvious in view of the cited art.

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

E. Fibach

Prof. Eitan Fibach

Signed this day 14 of April, 2004

Enc.:
Appendix A and B

TRA 1902839v4

first 4 patients on GS trial IND 10751

Gamida-Cell Ltd.

APPENDIX A

Patient #	1	2	3	4
Diagnosis	AML	ALL	HD	ALL
Age	21	17	24	10
Gender	Female	Male	Male	Male
Weight (kg)	50	75.1	77	53
Cell source	Un. of Colorado	Milan	Un. of Colorado	Un. Of Colorado

Units

Gamida-Cell Ltd.

APPENDIX A

	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4
Unit portions (%)	40/60	50/50	40/60	40/60
Viable MNCs in expanded portion ($x10^6$)	30	148	31	126
CD34+ cells in expanded portion ($x10^6$)	0.8	21.5	3.13	18
Fold expansion MNC	74	420.5	231	206
Fold expansion CD34	1.9	57.8	20.9	29.6

APPENDIX A

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1.1.1.1 In-process data

When the results of the endotoxin, sterility and mycoplasma tests are unacceptable, the manufacturing process is discontinued. The viability of the cells following selection with the CliniMACS should also meet specifications, however, proceeding with the manufacturing process is based on the transplanter medical judgment.

Test method	Specifications	Results*		
		Lot #BR004	Lot #BR005	Lot #BR006
Unit Portion (%)	-	40	50	40
Number of viable MNCs thawed ($x10^6$)	-	792	1675	476.7
% Viability - post thaw	-	85.3	85.5	98.7
Number of $AC133^+$ cells after column ($x10^4$)	-	42.5	37.2	15.4
% Viability - post CliniMACS	<50	54.8	17.4**	53.8
% Purity of $CD133^+$ cells (CD133PE/CD38FITC)	-	65.8	94.8	73.4
CFU per 1000 cells	-	76	245	130
Endotoxin content (Eu/ml) Day=0		<2.0	<2.0	<2.0
Sterility-Bacteria Day=0	No growth	No growth	No growth	No growth
Sterility-Fungal Day=0	No growth	No growth	No growth	No growth
Mycoplasma	Absence	Absent	Absent	Absent
Sterility-Bacteria Day=7	No growth	No growth	No growth	No growth
Sterility-Fungal Day=7	No growth	No growth	No growth	No growth
Endotoxin content (Eu/ml) Day=14		<2.0	<2.0	<2.0

* The processing of the manufacturing data for the batch to be transplanted into patient #4 is not yet complete, and therefore unavailable at the deadline for the submission of this report.

** The transplanter instructed the technicians to place cells into culture and to measure viability by 7AAD flow cytometry. The result by this method was 76.2%.

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1.1.1.2 Lot release data

Test method	Specifications	Results*		
		Lot #BR004	Lot #BR005	Lot #BR006
Total number of viable cells ($x10^6$)	-	30	148	31
% Viability	-	100	94.9	100
Fold Expansion of cells	-	74	420.5	231.3
% of $CD34^+$ cells	-	2.65	14.53	10.1
% of $CD34^+/CD38^-$ cells	-	0	0.34	0.84
% of $CD133^+$ cells	-	0.53	5.04	6.24
% of $CD133^+/CD38^-$ cells	-	0	0.27	0
CFU per 1500 cells	-	25.7	39	35.3
Gram stain	Negative	Negative	Negative	Negative
Endotoxin content (Eu/Kg)	<5 Eu/Kg/60min	3.96	2.77	2.68
Sterility-Bacteria	No growth	No growth	No growth	No growth
Sterility-Fungal	No growth	No growth	No growth	No growth
Mycoplasma	Absence	Absent	Absent	Absent

APPENDIX A

* The processing of the manufacturing data for the batch to be transplanted into patient #4 is not yet complete, and therefore unavailable at the deadline for the submission of this report.

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1.1.1.3 General lots details*

	Patient #001	Patient #002	Patient #003
Lot number	BR004	BR005	BROO6
Cell source	University of Colorado Cord Blood Bank	Milan Cord Blood Bank (Rome)	University of Colorado Cord Blood Bank
Start of Manufacturing	March 26, 2003	April 10, 2003	July 22, 2003
End of Manufacturing	Apr. 16, 2003	May 1, 2003	Aug 12, 2003
Patient administration	Apr. 16, 2003	May 1, 2003	Aug 12, 2003

* The processing of the manufacturing data for the batch to be transplanted into patient #4 is not yet complete, and therefore unavailable at the deadline for the submission of this report.

Israeli start-up Gamida-Cell to receive prize

Gamida-Cell, which is active in the field of blood stem cell expansion to treat disease, has raised $20 million to date.

Batya Feldman——5 Feb 04 16:54

Israeli start-up Gamida-Cell has been chosen to receive the "Best Abstracts Award" by the American Society for Blood and Marrow Transplantation. The award will be presented to the Gamida-Cell research team on February 13th in Orlando, Florida.

Gamida-Cell is active in the field of blood stem cell expansion for cancer and autoimmune disease therapies. The Gamida-Cell abstract describes test results in which the injection of expanded populations of human stem cells restored cardiac function in animals that had suffered a heart attack. Because of these results, the company has fast tracked plans to commence a Phase I/II study in 2004.

Gamida-Cell was founded in 1998. Since then, the company has raised $20 million in three rounds of financing. Shareholders include Mordechai (Motti) Zisser, Biomedical Investments, Teva Pharmaceuticals (Nasdaq:TEVA; TASE:TEVA), and venture capital funds Denali Ventures, Auriga Ventures, Pamot Rehovot Advisers' Pamot Venture Capital fund and Comverse subsidiary Comsor Venture Fund.

Gamida-Cell CEO Ehud Marom, a veteran in the healthcare field, is a former VP of Teva and Peptor, and one of the founders of Karma Pharm, which was acquired for $2 million by Biodar Pharma last month. Marom told "Globes" that last March, Gamida-Cell signed a strategic co-operation agreement with Teva (Nasdaq: TEVA). Under the agreement, Teva invested $3 million in Gamida-Cell, with an option for further investment once clinical trials were completed. Marom said this investment would be about $25 million, plus a 15% stake in the company.

Marom said that the company was now in a much better position to raise capital and sign strategic agreements. In the coming year, he said, Gamida-Cell would complete the first two phases of clinical trials, the goal being to realize the agreement with Teva. Marom also did not rule out the possibility of the company being acquired by a large corporation. He believes that if the market continues to be positive, major companies will seek out acquisitions. Gamida-Cell was certainly a good candidate, he said.

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APPENDIX B

HEALTH

FROM THE ARCHIVES: June 2, 2003

Stem-Cell Technology to Treat Leukemia Patients Shows Promise

By DAVID P. HAMILTON
Staff Reporter of THE WALL STREET JOURNAL

A phase I trial of StemEx, a technology from Gamida-Cell Ltd. for expanding populations of so-called hematopoietic stem cells, is off to a good start the company's chief executive said in an interview.

"The results are encouraging," said Gamida-Cell CEO Ehud Marom, without providing details. "It's a beginning, a good beginning."

Gamida-Cell, a closely held biotechnology firm based in Jerusalem, Israel, is testing StemEx in conjunction with the M. D. Anderson Cancer Center at the University of Texas in Houston.

The goal, Mr. Marom said, is to use StemEx to expand populations of so-called hematopoietic stem cells from stored umbilical-cord blood in order to reconstitute the immune systems of patients with leukemia or other blood cancers.

The process is similar to that involved in a bone-marrow transplant. In that procedure, doctors use chemotherapy and radiation to deliberately destroy the blood cells and immune system of leukemia patients, whose blood cells are multiplying out of control. Then an immunologically matched donor provides bone marrow containing hematopoietic stem cells, which can grow into a variety of different blood cells. When provided to the cancer patient, the cells reconstitute the patient's immune system, minus the cancer.

In this trial, physicians hope to use stem cells from cord blood, which some parents have stored shortly after the birth of their children, for those transplants. Cord blood contains a population of young stem cells that are thought to be particularly useful in transplantation.

Normally, however, cord blood doesn't yield enough stem cells to treat adult cancer patients. So doctors in the Houston trial will use StemEx, a chemical formulation that encourages the proliferation of stem cells, to "expand" populations of stem cells

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by as much as 100 to 1,000 times in culture. Animal studies have shown StemE- [illegible]e in not only expanding stem-cell populations, but in [illegible]ing better "grafts" leading to reconstituted immune systems, Gamida-Cell says.

The study should involve 10 patients, all over 55 years old, with a variety of blood cancers. Mr. Marom said the company hopes to have recruited all 10 [illegible]ients by the end of the year. If the trial goes well and the U.S. Food and Drug Administration approves, Mr. Marom said, the company hopes to move quickly to a larger, multi-center trial of about 80 patients.

Write to David P. Hamil[illegible] at david.hamilton@wsj.com

Updated June 12, [illegible]3

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